

Morphology and multigene phylogeny of *Talaromyces amyrossmaniae*, a new synnematus species belonging to the section *Trachyspermi* from India

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Abstract

A new *Talaromyces* species, *T. amyrossmaniae*, isolated from decaying fruit and litter of *Terminalia bellerica*, is described and illustrated. On the natural substrate, the new species produces determinate synnemata, with a well-defined, vivid orange red to orange red cylindrical stipe, and a greyish green capitulum. Conidiophores are typically biverticillate, or sometimes have subterminal branches, with acerose phialides that produce globose to subglobose, smooth to slightly roughened conidia. Multigene phylogenetic analyses based on the internal transcribed spacer region (ITS), and partial sequences of β -tubulin (*BenA*), calmodulin (*CaM*), and DNA directed RNA polymerase second large subunit (*RPB2*) genes, along with morphological characterization, revealed that these isolates are distinct and form a unique lineage of *Talaromyces* in section *Trachyspermi*, closely allied to *T. aerius*, *T. albobiverticillius*, *T. heiheensis*, *T. erythromellis*, and *T. solicola*. The new species *T. amyrossmaniae* is the first species in section *Trachyspermi* with determinate synnemata.

Keywords

BenA, *CaM*, conidial fungi, *RPB2*, synnemata, *Trichocomaceae*, Western Ghats

Introduction

The genus *Talaromyces* was described as a teleomorph-based holomorph genus (Benjamin 1955). It is characterized by cleistothecial ascomata that have a soft hyphal exterior giving them a yellow, cream, pink or reddish coloration; its anamorphs are predominantly biverticillate or rarely terverticillate conidiophores with acerose phialides with a narrow mouth (Samson et al. 2011, Yilmaz et al. 2014). Conventionally, species of *Talaromyces* were linked with *Penicillium*, *Paecilomyces*, *Geosmithia*, and *Merimbla* anamorphs (Pitt 1980, Pitt et al. 2000). Primary phylogenetic studies of *Talaromyces* spp. revealed that they form a distinct clade that includes species formerly classified in *Penicillium* subgenus *Biverticillium*, separate from *Eupenicillium* and *Penicillium* spp. in other subgenera (LoBuglio et al. 1993; Seifert et al. 1993; Berbee et al. 1995; Peterson 2000; Heredia et al. 2001; Seifert et al. 2004). As redefined following the new single name provision of the International Code of Nomenclature of algae, fungi and plants (ICN), *Talaromyces* was expanded to include asexual species formerly included in *Penicillium* subgenus *Biverticillium* (Samson et al. 2011; Visagie and Jacobs 2012; Visagie et al. 2012; Yilmaz et al. 2012, 2014). The landmark multigene phylogeny of *Penicillium* and allied genera by Houbraken and Samson (2011) segregated the prevailing concept of the family Trichocomaceae into three families, Aspergillaceae, Thermoascaceae, and Trichocomaceae. *Talaromyces* sensu stricto is presently classified in the Trichocomaceae along with *Thermomyces*, *Sagenomella*, *Rasamsonia*, and *Trichocoma*. The molecular taxonomy and nomenclature *Talaromyces* were comprehensively revised in the recent past (Houbraken and Samson 2011; Samson et al. 2011; Seifert et al. 2012; Visagie and Jacobs 2012; Visagie et al. 2012; Yilmaz et al. 2012; Yilmaz et al. 2014). Yilmaz et al. (2014) resolved the phylogenetic positioning of *Talaromyces* species using a polyphasic taxonomic concept and placing 88 accepted species in seven well-defined sections, namely *Bacillispori*, *Helici*, *Islandici*, *Purpurei*, *Subinflati*, *Talaromyces*, and *Trachyspermi*. Subsequent to the monograph by Yilmaz et al. (2014), 54 new *Talaromyces* species have been described from all over the world (Visagie et al. 2015; Chen et al. 2016; Luo et al. 2016; Crous et al. 2016; Romero et al. 2016; Wang et al. 2016; Wang et al. 2016; Yilmaz et al. 2016a, b; Crous et al. 2017; Guevara-Suarez et al. 2017; Peterson and Jurjević 2017; Wang et al. 2017; Barbosa et al. 2018; Su and Niu 2018; Jiang et al. 2018; Varriale et al. 2018).

During the 2009 monsoon season, routine surveys were conducted to explore microfungi diversity in natural forests of Lingmala waterfalls area (17.9218N; 73.6870E) of Mahabaleshwar, northern Western Ghats, India. A previously undescribed synnema-forming fungus with penicillate conidiophores and phialidic conidiogenous cells was collected from decaying fruits and litter of *Terminalia bellerica* (*Combretaceae*) fallen onto the ground near the Lingmala waterfalls. The fungus was isolated into pure culture on different culture media, microscopic characters were recorded and its classification studied using phylogenetic analysis of aligned DNA sequences from the nuclear ribosomal ITS region and *BenA*, *CaM*, and *RPB2* partial gene sequences. This paper aims to resolve the taxonomy and phylogeny of this synnematous species, which is shown to represent a new species in *Talaromyces* section *Trachyspermi*, here named *T. amyrossmaniae*.

Materials and methods

Isolation

Conidia were removed from synnemata directly from the surface of fallen fruits under a Nikon stereomicroscope (model SMZ1500 with Digital camera; Nikon, Tokyo, Japan) and placed on malt extract agar (MEA) media containing the antibiotic Streptomycin sulphate (100 mg/L) CMS220-5G (HIMEDIA Laboratories Pvt. Ltd, Mumbai, India). Methods and media used for examining colony characters, inoculating and incubating cultures, and microscopic examination followed those of Visagie et al. (2014), with the addition of Oatmeal Agar (OA), and Potato Dextrose Agar (PDA), with incubation occurring in a Bio Multi Incubator (Model LH-30-8CT, Japan). Herbarium specimens were deposited in the Ajrekar Mycological Herbarium (AMH); cultures were accessioned and preserved in the National Fungal Culture Collection of India (NFCCI; WDCM-932), Agharkar Research Institute, Pune, India. Reference and ex-type strains used in this study are listed in Table 1.

Morphology

Colony characters were recorded after 7 d of incubation on various media, including Czapek yeast autolysate agar (CYA), Blakeslee's (1915) malt extract agar (ME-Abl), yeast extract sucrose agar (YES), oatmeal agar (OA), and creatine sucrose agar (CREA). Bacto malt extract was used for MEAbl. Media preparation, inoculations, incubation conditions, and microscopic preparations followed the recommendations by Visagie et al. (2014). Colour codes and names used in descriptions are from Kornerup and Wanscher (1967). Microscopic observations were made with an Olympus (Model CX-41, Japan) dissecting microscope and Zeiss (AXIO Imager 2, Germany) compound microscope equipped with Nikon Digital sight DS-Fi1 and AxioCam MRc5 cameras driven by AxioVision Rel 4.8 software (AXIO Imager 2, Germany).

DNA extraction, amplification, and phylogenetic analyses

Colonies were grown on MEAbl plates, and genomic DNA was extracted following the rapid salt extraction method of Aljanabi and Martinez (1997). The ITS regions was amplified using primer pairs ITS5 and ITS4 (White et al. 1990). For the amplification of *RPB2* gene region, primer pairs RPB2-5F and RPB2-7cR (Liu et al. 1999) were used with touch-up PCR conditions: 5 cycles with annealing temperature 48 °C followed by 5 cycles at 50 °C and final 25 cycles at 52 °C. The partial *BenA* gene was amplified with primer pair Bt2a and Bt2b (Glass and Donaldson 1995) with 50 °C as annealing temperature. The partial *CaM* gene was amplified using primer pair CF1M

Table 1. Accession numbers for fungal strains and strains used for the phylogenetic analysis.

| Species | Collection no. | Substrate and origin | GenBank accession no. | | | |
|------------------------------|---------------------------|--|-----------------------|-------------|------------|-------------|
| | | | ITS | <i>BenA</i> | <i>CaM</i> | <i>RPB2</i> |
| <i>T. aerius</i> | CBS 140611 ^T | Indoor air, China | KU866647 | KU866835 | KU866731 | KU866991 |
| <i>T. albobiverticillius</i> | CBS 133440 ^T | Decaying leaves of a broad leaved tree, Taiwan | HQ605705 | KF114778 | KJ885258 | KM023310 |
| <i>T. amyrossmaniae</i> | CBS 140498 | Air from HVAC system, China | KR855658 | KR855648 | KR855653 | KR855663 |
| | NFCCI 1919 ^T | Fallen decaying fruits of <i>Terminalia bellerica</i> (Combretaceae), Maharashtra, India | MH909062 | MH909064 | MH909068 | MH909066 |
| | NFCCI 2351 | Fallen decaying fruits of <i>Terminalia bellerica</i> (Combretaceae), Maharashtra, India | MH909063 | MH909065 | MH909069 | MH909067 |
| <i>T. assiutensis</i> | CBS 147.78 ^T | Soil, Egypt | JN899323 | KJ865720 | KJ885260 | KM023305 |
| | CBS 645.80 | <i>Gossypium</i> , India | JN899334 | KF114802 | * | * |
| <i>T. atroroseus</i> | CBS 133442 ^T | House dust, South Africa | KF114747 | KF114789 | KJ775418 | KM023288 |
| | CBS 133449 | Mouse dung, Denmark | KF114744 | KF114788 | * | * |
| <i>T. austrocalifornicus</i> | CBS 644.95 ^T | Soil, USA | JN899357 | KJ865732 | KJ885261 | * |
| <i>T. brasiliensis</i> | CBS 142493 ^T | Honey of <i>Melipona scutellaris</i> ; Recife, Pernambuco, Brazil | MF278323 | LT855560 | LT855563 | LT855566 |
| <i>T. convolutus</i> | CBS 100537 ^T | Soil, Nepal | JN899330 | KF114773 | * | JN121414 |
| <i>T. diversus</i> | CBS 320.48 ^T | Leather, USA | KJ865740 | KJ865723 | KJ885268 | KM023285 |
| | DTO 244-E6 | House dust, New Zealand | KJ775712 | KJ775205 | * | * |
| <i>T. erythromellis</i> | CBS 644.80 ^T | Soil from creek bank, New South Wales | JN899383 | HQ156945 | KJ885270 | KM023290 |
| <i>T. heiheensis</i> | HMAS 248789 ^T | Rotten wood, China | KX447526 | KX447525 | KX447532 | KX447529 |
| <i>T. minioluteus</i> | CBS 642.68 ^T | Unknown | JN899346 | KF114799 | KJ885273 | JF417443 |
| | CBS 270.35 | <i>Zea mays</i> , USA | KM066172 | KM066129 | * | * |
| | CBS 137.84 | Fruit damaged by insect, Spain | KM066171 | KF114798 | * | * |
| <i>T. minnesotensis</i> | CBS 142381 ^T | Human ear, USA | LT558966 | LT559083 | LT795604 | LT795605 |
| <i>T. solicola</i> | DAOM 241015 ^T | Soil, South Africa | FJ160264 | GU385731 | KJ885279 | KM023295 |
| | CBS 133446 | Soil, South Africa | KF114730 | KF114775 | * | * |
| <i>T. systylus</i> | BAFCcult3419 ^T | Soil, Argentina | KP026917 | KR233838 | KR233837 | * |
| <i>T. trachyspermus</i> | CBS 373.48 ^T | Unknown, USA | JN899354 | KF114803 | KJ885281 | JF417432 |
| | CBS 118437 | Soil, Morocco | KM066169 | KM066127 | * | * |
| <i>T. ucrainicus</i> | CBS 162.67 ^T | Unknown | JN899394 | KF114771 | KJ885282 | KM023289 |
| | CBS 127.64 | Soil treated with cyanimide, Germany (ex-type of <i>T. ohiensis</i>) | KM066173 | KF114772 | * | * |
| <i>T. udagawae</i> | CBS 579.72 ^T | Soil, Japan | JN899350 | KF114796 | KX961260 | * |

^T: ex-type strain

and CF4 (Hubka et al. 2014) subjected to 32 cycles under the following temperature regime: first cycle at 95 °C for 3 min, 55 °C for 30 seconds, and 72 °C for 1 min; followed by 30 cycles at 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 1 min; and a final cycle at 95 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 10 min. PCR products were purified with StrataPrep PCR Purification Kit (Agilent Technologies, TX, USA) and sequenced using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequencing reactions were run on a ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, USA).

Sequence alignment and phylogenetic analysis

Reference sequences of *Talaromyces* section *Trachyspermi* were downloaded from GenBank and aligned in MAFFT v. 7.305b (Kato and Standley 2013) with the newly generated sequences. Alignments were manually adjusted in Geneious as needed. A Maximum Likelihood analysis was done in IQtree v. 1.6 (Nguyen et al. 2015) after selecting the most suitable substitution model with the Modelfinder (Kalyaanamoorthy) algorithm built into the software. The trees were visualized in Figtree v. 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree>) and edited for publication in Affinity Designer v. 1.6.1 (Serif Europe Ltd, UK). The new DNA sequences were deposited in GenBank (Table 1).

Results

Phylogenetic analyses

The phylogenetic analysis showed that the new species described below as *Talaromyces amyrossmaniae* belongs to section *Trachyspermi*. The relationships of the new species with accepted species and its genetic coherence and phylogenetic consistency were analysed with single concatenated sequence datasets based on four loci (ITS, *BenA*, *CaM* and *RPB2*). The length of the data sets were 540 bp for ITS, 379 bp for *BenA*, 550 bp for *CaM*, and 851 bp for *RPB2* loci. The best fitting models for the ML analysis were TPM2u+F+I+G4 for ITS, TIM2e+G4 for *BenA*, K2P+I+G4 for *CaM*, and K2P+I+G4 for *RPB2*. All trees were rooted with *T. pinophilus* (CBS 631.66). The single gene trees and the multigene phylogram are shown in Figures 1, 2.

Because of the limited resolution of the official fungal DNA barcode, the ITS (Schoch et al. 2012), in the *Trichocomaceae*, *BenA* was proposed as the secondary DNA barcode for *Talaromyces* (Yilmaz et al. 2014). The overall tree topologies of ITS and *BenA* phylogenies had relatively consistent association of species. However, the type species of section *Trachyspermi*, *T. trachyspermus* was well separated from *T. assiutensis* in the *BenA* analysis whereas strains of the two species were intermixed in the ITS analysis. *Talaromyces ucraicinus* was consistently a sister clade to *T. trachyspermus* and *T. assiutensis*. Our proposed new species, *T. amyrossmaniae*, was distinguished from other species both by ITS and other markers (Figs 1, 2). It is consistently included in a major clade along with *T. aerius*, *T. albobiverticillius*, *T. erythromellis*, *T. heiheensis*, and *T. solicola* in the ITS analysis. As with the ITS, in the concatenated phylogeny and *RPB2* analyses, *T. amyrossmaniae* clustered with *T. albobiverticillius*, *T. heiheensis*, *T. erythromellis*, *T. aerius*, and *T. solicola* (Figs 1, 2). However, in the *BenA* phylogeny, *T. amyrossmaniae* was segregated from that major-clade. *Talaromyces amyrossmaniae* is clustered with *T. austrocalifornicus* in the *CaM* analyses (Fig. 1). Yilmaz et al. (2014) mentioned that amplification of *CaM* is difficult in section *Trachyspermi*. *CaM* data

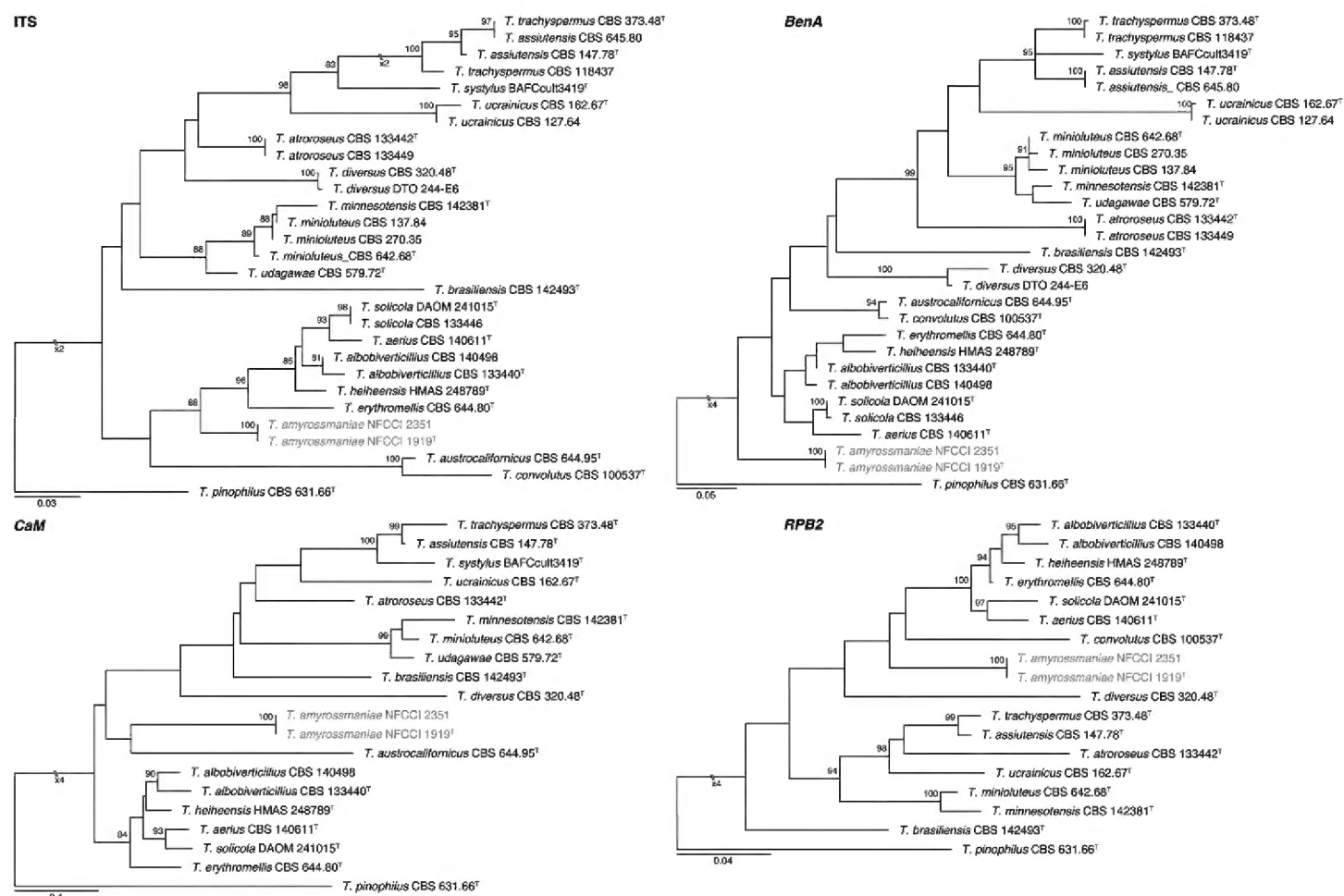


Figure 1. Maximum likelihood (ML) phylogenetic trees of ITS region, *BenA*, *CaM* and *RPB2* genes of strains belong *Talaromyces* section *Trachyspermi*. *Talaromyces pinophilus* (CBS 631.66^T) was chosen as outgroup. Bootstrap values above 70% are indicated. Purple names indicate *T. amyrossmaniae* strains. ^T: ex-type.

could not be analyzed critically because the new sequences generated through Sanger sequencing (ABI PRISM 3100 Genetic Analyzer) contained a homopolymer stretch of around 30 bp after base 320, resulting in poor quality and short sequences, even after many attempts using modified PCR conditions and primers.

Morphology

In this study, we introduce one new species, *Talaromyces amyrossmaniae* belonging to section *Trachyspermi*. Strains conform with the general morphological characters of this section. *Talaromyces amyrossmaniae* was compared with its close relatives, with the distinguishing characters mentioned in the note after the species description. Also, Table 2 compares the new species with the closely allied species in section *Trachyspermi*. The main character that differentiates *T. amyrossmaniae* from other synnemata-producing species in the genus *Talaromyces* is the length of the synnemata. *Talaromyces amyrossmaniae* has the shortest synnemata (up to 150 µm). A synopsis of comparative morphology and growth rate of synnema producing species of *Talaromyces* is given in Table 3.

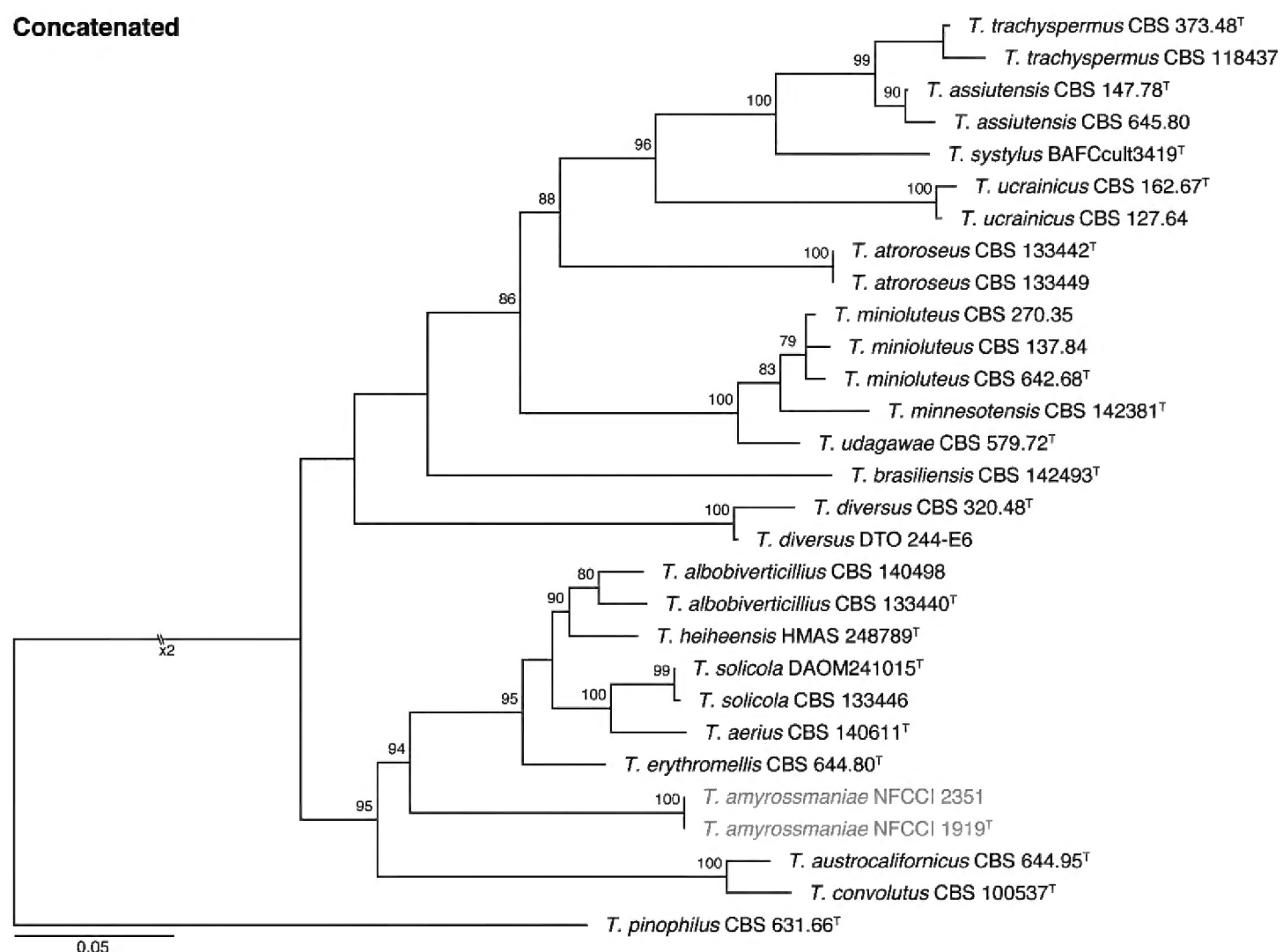
Concatenated

Figure 2. Maximum likelihood (ML) combined phylogenetic trees using ITS region, *BenA*, *CaM* and *RPB2* genes of strains belong *Talaromyces* section *Trachyspermi*. *Talaromyces pinophilus* (CBS 631.66^T) was chosen as outgroup. Bootstrap values above 70% are indicated. Purple names indicate *T. amyrossmaniae* strains. ^T: ex-type.

Taxonomy***Talaromyces amyrossmaniae* Rajeshkumar, Yilmaz & Seifert, sp. nov.**

Mycobank MB518601

Figure 3

Etymology. Latin, named after Dr Amy Y. Rossman, in honour of her career as a research leader in Systematic Mycology and Microbiology, USDA ARS, Beltsville, Maryland, USA.

Diagnosis. Synnemata abundant in nature, determinate, 90–120 µm tall, with an unbranched stalk 10–35 µm wide, base wider, up to 50–60 µm. Synnema stipe orange red or vivid orange red, capitulum terminal, compact, globose with conidiophores and a powdery grey-green conidial mass in closely packed, split columns. On MEAbI synnemata produced after 2 weeks incubation, up to 120 µm long. Conidiophores biverticillate, with sometime terverticillate sub-branches. Acerose phialides producing

Table 2. Comparative morphology of *Talaromyces* section *Trachyspermi*.

| Species | Conidiophore branching | Conidia ornamentation | Conidial shape | Conidial size (µm) |
|------------------------------|--|-----------------------------------|--------------------------------|----------------------------------|
| <i>T. aerius</i> | Biverticillate, minor proportion with subterminal branches | Smooth | Ellipsoidal | 2–3.5 (–4.5) × 2–3 |
| <i>T. albobiverticillius</i> | Biverticillate, minor proportion with subterminal branches | Smooth to finely roughened | Globose to subglobose | 2–3.5 (–4) × 1.5–2.5 |
| <i>T. amyrossmaniae</i> | Biverticillate, minor proportion with subterminal branches | Smooth to finely roughened | Globose or subglobose | 2.5–4 (–6) × 2.5–3.5 (–8) |
| <i>T. assiutensis</i> | Mono to biverticillate | Smooth | Ovoidal to ellipsoidal | 2–4 × 1.5–2.5 |
| <i>T. atroroseus</i> | Biverticillate, minor proportion with subterminal branches | Finely roughened to rough | Ellipsoidal | 2–3.5 × 1.5–2.5 |
| <i>T. austrocalifornicus</i> | Biverticillate | Smooth | Subglobose | 1.5–3 × 1.5–2.5 |
| <i>T. brasiliensis</i> | Biverticillate | Finely roughened | Globose | 2 × 3 |
| <i>T. convolutus</i> | Mono to biverticillate | Smooth | Ellipsoidal | (2–) 3–4 × 1.5–2 (–3) |
| <i>T. diversus</i> | Biverticillate, minor proportion with subterminal branches | Smooth to finely roughened | Subglobose to ellipsoidal | 2–3 (–5) × 2–3 (–3.5) |
| <i>T. erythromellis</i> | Biverticillate having symmetrical subterminal branches, | Smooth | Subglobose to ellipsoidal | 2–3.5 × 1.5–2.5 |
| <i>T. heiheensis</i> | Biverticillate with subterminal branches, minor proportionquaterverticillate | Smooth | Subglobose to ellipsoidal | 2.5–3 × 2–2.5 |
| <i>T. minioluteus</i> | Biverticillate | Smooth | Ellipsoidal | 2.5–4 × 1.5–2.5 |
| <i>T. minnesotensis</i> | Biverticillate | Smooth | Ellipsoidal | 2.5–3.5 × 2–3 |
| <i>T. solicola</i> | Biverticillate | Rough | Globose to subglobose | 2–3.5 × 2–2.5 |
| <i>T. systylus</i> | Biverticillate | Rough | Globose | 3.5 × 4 |
| <i>T. trachyspermus</i> | Mono to biverticillate | Smooth | Ellipsoidal | 2–3.5 (–5) × 1.5–2.5 |
| <i>T. ucrainicus</i> | Mono to biverticillate | Smooth | Broadly ellipsoidal to ovoidal | 2–4 (–5) × 1.5–2.5 (–3) |
| <i>T. udagawae</i> | Biverticillate | Smooth | Subglobose to ellipsoidal | 3–4 × 2–3 |

smooth to slightly roughened globose to subglobose conidia. Restricted growth on all media, acid production absent on CREA.

In: *Talaromyces* section *Trachyspermi*.

Type. INDIA, Maharashtra, Mahabaleshwar, Lingmala falls; isolated from fallen decaying fruits and litter of *Terminalia bellerica* (*Combretaceae*), 9 June 2009, isolated by K.C.Rajeshkumar, holotype: AMH 9330, extype: NFCCI 1919, other culture NFCCI 2351.

Gene sequences: ex-holotype MH909062(ITS), MH909064(*BenA*), MH909068(*CaM*), MH909066(*RPB2*).

Description. Colony diameter, 7 d (mm): CYA 4–6; CYA 37 °C no growth; ME-Abl 12–14; YES 5–7; DG18 4–5; OA 10–13; CREA 3–5.

Colony characters: CYA 25 °C, 7 d: colonies low, plane; margins low, entire (< 1 mm); mycelia white; no germination; sporulation absent; soluble pigmentation absent;

Table 3. Synopsis of comparative morphology and growth rate of synnema producing species of *Talaromyces*.

| Species | Section | Synnemata | | | Growth rates (mm) | | | |
|--|---------------------|--|--------------------|-------------|-------------------------|-----------|-----------|-----------|
| | | Shape | Time of production | Height (µm) | Acid production on CREA | CYA 25 °C | CYA 37 °C | MEA 25 °C |
| <i>T. amyrossmaniae</i> ^a | <i>Trachyspermi</i> | Determinate | Prolonged | Up to 150 | Absent | 4–6 | No growth | 12–14 |
| <i>T. calidicanius</i> ^b | <i>Talaromyces</i> | Determinate | Prolonged | Up to 6000 | Moderate | 27–30 | No growth | 47–48 |
| <i>T. cecidicola</i> ^b | <i>Purpurei</i> | Determinate | Prolonged | Up to 1250 | Absent | 33–34 | No growth | 37–38 |
| <i>T. choloroloma</i> ^b | <i>Purpurei</i> | Determinate | Prolonged | Up to 1200 | Weak to moderate | 40–45 | No growth | 45–48 |
| <i>T. coalescens</i> ^b | <i>Purpurei</i> | Determinate | Prolonged | Up to 1200 | Very weak | 32–34 | 2–4 | 43–45 |
| <i>T. dendriticus</i> ^b | <i>Purpurei</i> | Determinate | Prolonged | Up to 5000 | Absent | 23–26 | 5–6 | 35–36 |
| <i>T. duclauxii</i> ^b | <i>Talaromyces</i> | Indeterminate | After 7d | Up to 5000 | Weak | 25–27 | 3–4 | 48–50 |
| <i>T. flavovirens</i> ^b | <i>Talaromyces</i> | Determinate, covered or masked by yellow mycelial covering | Prolonged | Up to 750 | Absent | 19–20 | 5–6 | 37–38 |
| <i>T. palmae</i> ^b | <i>Subinflati</i> | Indeterminate | Prolonged | Up to 8000 | Weak | 20–25 | No growth | 22–26 |
| <i>T. panamensis</i> ^b | <i>Talaromyces</i> | Determinate, cone shaped and often sterile | After 7d | Up to 6800 | Strong | 23–24 | No growth | 28–30 |
| <i>T. pittii</i> ^b | <i>Purpurei</i> | Determinate, phototropic | Prolonged | Up to 1000 | Absent | 34–36 | No growth | 42–44 |
| <i>T. pseudostromaticus</i> ^b | <i>Purpurei</i> | Determinate | Prolonged | Up to 8000 | Absent | 25–34 | No growth | 38–43 |
| <i>T. ramulosus</i> ^b | <i>Purpurei</i> | Determinate | Prolonged | Up to 500 | Absent | 32–40 | 5–8 | 45–48 |
| <i>T. systylus</i> ^c | <i>Trachyspermi</i> | Indeterminate | Prolonged | Up to 4000 | Good | 14–18 | 16–19 | 18–21 |

^a: Data from this study. ^b: Data from Yilmaz et al. (2014). ^c: Data from Romero et al. (2016).

exudates absent; reverse Yellowish white (4A2). MEAb 25 °C, 7 d: colonies low, slightly raised, synnemata present; margins low, entire (1 mm); mycelia white; texture velvety; sporulation dense (except margins); conidia en masse Dull green (27D4–27E4); soluble pigmentation yellow; exudates orange to reddish orange small droplets; reverse Hazel brown (6E6) at center fading into Light brown (6D8) to Light yellow (3A5). YES 25 °C, 7 d: colonies slightly raised, sulcate, sunken at center; margins low, entire (< 1 mm); mycelia pale pinkish red, with and appearance of Pastel red (7A4–7A5); texture floccose; sporulation absent; soluble pigmentation absent; exudates absent; reverse Light brown (7D6). DG18 25 °C, 7 d: colonies slightly raised, sulcate, sunken at center; margins low, entire (1 mm); mycelia pale yellow; texture floccose; sporulation moderately dense at center, margins absent; conidia en masse Greyish green to Dull green (26C4–26D4); soluble pigmentation absent; exudates absent; reverse Brownish orange to Brownish yellow (5C6) in the center, fading into Light yellow (4A5). OA 25 °C, 7 d: colonies low, plane; margins low, entire (< 1 mm); mycelia white; texture velvety; sporulation moderately dense at center, margins absent; conidia en masse Greyish green (27C4–27D4); soluble pigmentation dark red; exudates absent; reverse Brown (7E8) in the centre, fading into Copper red (7C8). CREA 25 °C, 7 d: acid production absent.

Micromorphology. Determinate synnemata formed after 2 weeks on MEAb up to 80–150 µm long. Conidiophores biverticillate with a minor proportion having subterminal branches; stipes smooth walled 80–120 × 3–4 µm; extra branches up to 30 µm long;

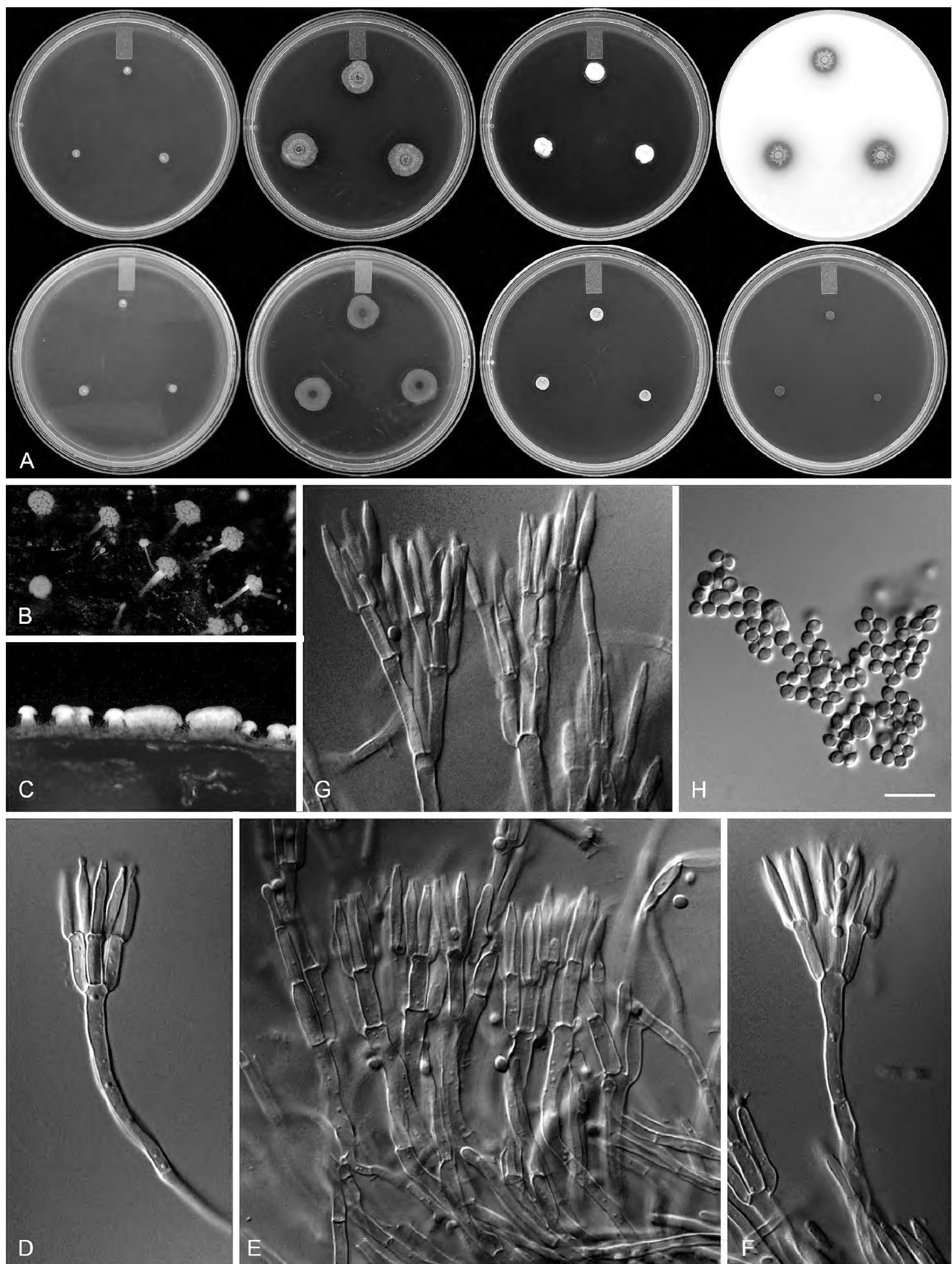


Figure 3. *Talaromyces amyrossmaniae* (NFCCI 1919) **A** Colonies on CYA, MEAbI (obverse and reverse), Colonies obverse on YES, OA, DG18, CREA **B** Synnemata on *Terminalia bellerica* fruit in nature **C** Synnema formation on MEAbI after 14 d at 25 °C **D–F** Biverticillate penicilli **G** Biverticillate penicilli with subterminal branches **H** Conidia. Scale bar: 10 µm.

metulae three to six, divergent, $10\text{--}13 \times 2.5\text{--}3 \mu\text{m}$; phialides acerose, three to six per metulae, $12\text{--}15$ ($\text{--}18$) $\times 2\text{--}3 \mu\text{m}$; conidia smooth, globose to subglobose, $2.5\text{--}4 \times 2.5\text{--}3.5 \mu\text{m}$. Sometimes it produces large-sized conidia up to $6\text{--}8 \mu\text{m}$. Ascomata not observed.

Discussion

In our study, a novel *Talaromyces* species, *T. amyrossmaniae* is described based on two isolates from decaying fruits and litter of *Terminalia bellerica* (*Combretaceae*). We used ITS, *BenA*, *CaM* and *RPB2* sequences to apply genealogical concordance phylogenetic species recognition (GCPSR; Taylor et al. 2000) to delineate the species, and a multi-gene phylogenetic analysis to place *T. amyrossmaniae* in *Talaromyces* section *Trachyspermi*. *Talaromyces* section *Trachyspermi* (as '*trachyspermus*') was introduced by Yaguchi et al. (1996) based on ubiquinone systems, overriding the traditional morphology based classification of *Talaromyces*. Yilmaz et al. (2014) applied multigene phylogenies and morphology to redefine classification of *Talaromyces* and divided the genus into seven sections. They noted that *Talaromyces* section *Trachyspermi* includes species with generally biverticillate penicilli with acerose phialides and when ascomata are produced, they are creamish white or yellow. Colonies generally grow restrictedly on CYA, YES, CREA and DG18; some species have colonies with abundant red pigments.

Morphologically, *T. amyrossmaniae* resembles the other species of section *Trachyspermi* and produces dark orange to red pigmentation on MEAbI, restricted growth on MEAbI, CYA, DG18, YES and CREA, symmetrical biverticillate penicilli with a minor proportion having sub-terminal branches, and acerose phialides that form globose to subglobose, smooth to slightly roughened conidia. Although, synnematus *Talaromyces* species also are found in section *Purpurei*, section *Talaromyces*, section *Trachyspermi*, and section *Subinflati*, *T. amyrossmaniae* is the first species in section *Trachyspermi* with determinate synnemata that are seen on fallen decaying fruits in nature and also on MEAbI after 7–14 d of incubation at 25 °C. *Talaromyces systylus* is another synnema producer in section *Trachyspermi*, but it produces indeterminate synnemata up to 4000 μm and grows at 37 °C (Romero et al. 2016). *Talaromyces amyrossmaniae* has the shortest synnemata in *Talaromyces* and it grows very restrictedly compared to the other synnema producing species on CYA and MEAbI. With these key characters, it is easy to distinguish the new species from the other synnemata producing species of *Talaromyces*.

Based on ITS, *BenA*, *CaM*, and *RPB2* phylogenies, *T. amyrossmaniae* is part of the same clade as *T. austrocalifornicus*, *T. convolutus*, *T. heiheensis*, *T. aerius*, *T. solicola*, *T. albobiverticillius*, and *T. erythromellis*; however, it can be distinguished from all of these species by having determinate synnemata in nature and by differences in colony growth characteristics. Also, *T. amyrossmaniae* forms predominant concentric rings of synnemata on the different media used in our studies and even forms synnemata in vitro on MEAbI.

Many species of *Talaromyces* have been recorded as saprophytes, endophytes, and human pathogens from different geoclimatic regions and microhabitats across India. Most importantly, *Talaromyces marneffe* is a potentially pathogenic thermally dimorphic fungus causing systemic mycosis in HIV-infected patients; its dissemination was thoroughly studied from Manipur state of India (Singh et al. 1999; Ranjana et al. 2002). Recent studies on endophytic *T. pinophilus* isolated from the rhizomes of *Curcuma amada* from Karnataka revealed the production and partial characterization of L-asparaginase (Krishnapura and Belur 2016). Likewise, endophytic *T. radicus*, isolated from *Catharanthus roseus*, produces vincristine and vinblastine and was studied for induce apoptotic cell death (Palem et al. 2015). *Talaromyces flavus* is also recorded as an endophytic fungi isolated from ethno-medicinal plants in the sacred forests of Meghalaya having antimicrobial and antioxidant activity (Bhagobaty and Joshi 2012). Devi et al. (2014) reported a marine strain of *T. verruculosus*, from Andhra Pradesh, as a potent polyhydroxybutyrate degrader. Similarly, the stress-tolerant soil fungus *T. funiculosus*, isolated from the neem rhizosphere, was identified as a potential strain for phosphate solubilization (Kanseet al. 2015). *Talaromyces flavus* isolated from paddy rhizosphere of Darjeeling Hills exhibited phosphate solubilizing activity in vitro and positively influenced the growth of *Oryza sativa*, *Cicer arietinum*, and *Vigna radiata* under greenhouse conditions (Chakraborty et al. 2011). A keratin degrading strain of *T. trachyspermus* was isolated from the grounds of a gelatin factory in Jabalpur, Madhya Pradesh, and digested human hair in stationary culture (Rajak et al. 1991). *Talaromyces trachyspermus* was reported as a soil saprophyte in paddy fields of Orissa (Dutta and Ghosh 1965). Species identifications of these *Talaromyces* strains were mostly based on micro- and macro morphological characters in these studies. Because such approaches often underestimate species diversity, adoption of a polyphasic approach to authenticate such identifications will increase the number of *Talaromyces* species known from different eco-geographic zones of India. Further investigation is also needed to study the ecological importance of these species.

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